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Inheritance and development of reduced and elevated palmitate in mutant lines of soybean

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Inheritance and development of reduced and elevated palmitate in mutant lines of soybean

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ABSTRACT

The saturated fatty ester content of soybean [*Glycine max* (L.) Merr.] oil may influence its use in food products. The palmitate content in the seed oil of common soybean cultivars is ~ 110 g kg^{-1}. Soybeans with reduced or elevated palmitate have been developed.

An inheritance study was conducted to determine if the *fapx* allele for reduced palmitate in A22 was at the same locus as *fap2* in C1727 and if the *fap?* allele in A24 for elevated palmitate was at the same locus as *fap1*. The F_2 segregation ratios and the segregation of F_3 seeds from the F_2 plants indicated that A22 and A24 have alleles at different independent loci than those of C1726, C1727, and A21. The temporary designation of *fapx* for the allele in A22 was replaced by the permanent designation of *fap3*, and the temporary designation of *fap?* for the allele in A24 was replaced by the permanent designation of *fap4*. The study showed that there are at least four independent loci in soybean that control palmitate content.

It would be useful in a breeding program to be able to distinguish between normal, reduced, and elevated palmitate genotypes before the flowering of a plant is complete or the seed is mature. A separate experiment was conducted to

(a) determine the earliest stage of seed development at which genotypes with normal, reduced, and elevated palmitate could be differentiated and (b) determine the relationship between the fatty ester content of the seed and that of the vegetative tissues of the three palmitate genotypes. It was determined that differences among reduced, elevated and normal palmitate lines can be detected as early as 17 DAF and remain throughout seed development. Significant differences for palmitate content of roots, stems, and leaves were observed among the reduced, elevated and normal palmitate genotypes as early as V2, the first-trifoliate leaf stage. The study also indicated that the genes for reduced, elevated, and normal palmitate are constitutively expressed.

GENERAL INTRODUCTION

Introduction

Soybeans [*Glycine max* (L.) Merr.] are a major source of edible fats and oils in the world. In the United States alone, more than 12,000 million pounds of soybean oil were utilized in 1994, of which 94% was for food purposes (Soya Bluebook, 1995). Some major soybean oil products include shortening, margarine, cooking oil, and salad oil.

The fatty ester composition in the seed oil is an important factor in determining the quality of soybean oil. Soybean oil consists of five major fatty esters: palmitate (16:0), stearate (18:0), oleate (18:1), linoleate (18:2), and linolenate (18:3). The fatty esters are distinguished by the number of carbons and double bounds in the fatty ester chain. For example, palmitate has 16 carbons in the fatty ester chain with zero double bonds, whereas linolenate has 18 carbons with three double bonds. Each of the fatty esters can be classified as either saturated, monounsaturated, or polyunsaturated (Table 1).

The oil processors refine and modify crude soybean oil for specific products by bleaching, deodorizing, and hydrogenating the oil (White, 1992). Hydrogenation is used to elevate the saturated fatty ester content of the oil to

Table 1. Classification and composition of commercial soybean oil.

Classification	Fatty ester		Composition
			g kg^{-1}
Saturated	Palmitate	(16:0)	110
	Stearate	(18:0)	40
Monounsaturated	Oleate	(18:1)	240
Polyunstaturated	Linoleate	(18:2)	540
	Linolenate	(18:3)	70

produce plastic fat products, that remain in a solid state at room temperatures, such as margarine and shortening. Hydrogenation is also used to stabilize cooking and salad oils by reducing the amount of linolenate in the oil. However, this process leads to the formation of trans-fatty esters in hydrogenated vegetable oils. In natural oils, the unsaturated fatty esters are in the cis configuration. It has been suggested in recent dietary studies that trans-fatty esters in the diet may have anti-nutritional qualities (White, 1992).

Although fatty esters are essential to the human diet, it has been suggested that diets high in saturated fat may lead to elevated cholesterol levels in the blood plasm, which contributes to several forms of cardiovascular diseases (Nelson, 1992). This information has led the U.S. government

to require that the food industry to provide information on the saturated fat content on the label. In addition, there are specific guidelines that the food industry must meet to label their products "reduced or low saturated fat". For example, a product labeled "low saturated fat" it must have less than 1 g of total saturated fat per 14 g serving. For soybean oil to meet that requirement, it would have to contain less than 71 g kg^{-1} of total saturated fatty esters in a 14 g serving of oil. Current soybean cultivars have about 150 g kg^{-1} total saturated fat, therefore, the oil from these cultivars cannot be utilized for the low saturates market.

It has been the goal of several breeding programs to develop soybean cultivars that would enhance the nutritional and functional quality of the oil. Soybeans with modified fatty ester content have been reported (Fehr et al., 1991 a,b; Fehr et al., 1992; Graef et al., 1985). The ranges of fatty ester content that has been developed in soybean are shown in Table 2. Chemical mutagenesis was used to create genetic variation for fatty ester content

Hammond and Fehr (1983 a,b; 1991a,b) have used the chemical mutagenic agents ethyl methanesulfonate (EMS), N-nitroso N-methyl urea (NMU), and sodium azide (SA) to generate new fatty ester mutants. Some of the mutants that have been

Table 2. Range of fatty ester content for soybean mutants with modified fatty ester composition in the seed oil.

Fatty Ester	Range of Composition
	g kg^{-1}
Palmitate (16:0)	< 35 - > 280
Stearate (18:0)	< 25 - > 280
Linolenate (18:3)	< 20

(Source: Erickson et al., 1988 Fehr et al., 1991 a,b; Fehr et al., 1992; Graef et al., 1985)

generated include elevated palmitate, reduced palmitate, reduced linolenate, and elevated stearate. Wilcox et al. (1984) used EMS on the cultivar 'Century' and developed fatty ester mutants.

Further reductions in fatty ester content have been made by intermating fatty ester mutants. The line A18 (< 40 g kg^{-1} palmitate) was developed by crossing A22 (≈ 78 g kg^{-1} palmitate) with C1726 (≈ 85 g kg^{-1} palmitate) (Fehr et al., 1991a). C1726 has the *fap1* allele for reduced palmitate (Erickson et al., 1988). A22, previously designated A1937NMU-173, has an allele for reduced palmitate that was given the temporary designation of *fapx* because its relationship to the *fap2* locus was not known (Fehr et al.,1991a).

The line A19 (> 280 g kg^{-1} palmitate) was developed by crossing A21 (≈ 200 g kg^{-1} palmitate) with A24 (≈ 180 g kg^{-1}

palmitate). A21, previously designated A1937NMU-85, has an allele (*fap2-b*) for elevated palmitate content (Fehr et al. 1991b) at the same locus or a tightly linked locus as the *fap2* allele in C1727 (Erickson et al. 1988). A24, previously designated ElginEMS-421, has an allele controlling elevated palmitate that was given the designation of *fap?* because the relationship to the *fap1* locus was not known.

One objective of my research, which is described in chapter 2, was to determine if the *fapx* allele in A22 was at the same locus as *fap2* and if the *fap?* allele in A24 was at the same locus as *fap1*.

It would be useful in a breeding program to be able to distinguish between normal, reduced, and elevated palmitate genotypes before flowering of a plant is complete or before seed is mature. Selection before the termination of flowering would make it possible to select and cross individuals during the same season. Selection before maturity could reduce the number of plants harvested and threshed and could shorten the time between harvest, selection, and replanting of individuals. The second objective of my study, which is presented in chapter 3, were to determine the stage of seed development at which genotypes with different palmitate contents can be distinguished and to compare the palmitate content of roots, stems, leaves, and mature seeds.

Dissertation Organization

The dissertation includes four chapters. Chapter 1 includes a literature review of the relevant studies which pertain to the experiments presented in the dissertation. Chapter 2 is a study of inheritance of reduced and elevated palmitate in mutant lines of soybean, which has been published in the journal, Crop Science. Chapter 3 is the study of fatty ester development in reduced and elevated palmitate lines of soybean, which has been submitted for publication in the journal, Crop Science. The tables for each of the experiments are presented at the end of each chapter. The general conclusions for the two experiments follow chapter 3. A comprehensive list of references included in the dissertation follow the general conclusions.

CHAPTER 2. LITERATURE REVIEW

Inheritance of Fatty Ester Composition in Mutant Soybean Lines

Knowledge of the inheritance of a trait, such as fatty ester composition, is important to the breeder to effectively design a cultivar development program. Traits of importance to a crop can be broadly defined as being either qualitative or quantitative. Qualitative traits are those controlled by one or a few major genes. Quantitative traits are controlled by several genes and expression may vary widely due to environment. Allard (1964) suggested that "the differences between qualitative and quantitative traits is not absolute". Environmental effects can significantly enhance the complexity of the inheritance of a trait whether it is controlled by a single gene or several genes.

The heritability of a trait in the broad sense is defined as $H=\delta^2_g/\delta^2_g+\delta^2_e$, where δ^2_g is the genetic variance and δ^2_e is the variance due to environment. Therefore, the heritability of a trait is inversely related to the influence of environment on the trait. For example, if a trait is controlled by a single major gene, but expression is influenced by environment, the distribution of the population may resemble that of a quantitative character. In addition, as the number of genes influencing a trait increases, the theoretical distribution of the population becomes normalized

without regard to the effects of the environment(Allard, 1964).

When new traits, such as fatty ester composition, are identified, it is important to conduct the appropriate study to determine the inheritance of the trait. The inheritance study will provide valuable information about the number of genes involved, level of dominance, and if any maternal or cytoplasmic effects are present.

The inheritance of genes controlling reduced and elevated palmitate content in soybean have been reported. Bubeck et al. (1989) studied the inheritance of four mutants that had a range of 128 to 182 g kg^{-1} palmitate content. They reported that the elevated palmitate in each of the mutants was controlled by alleles at a single major locus. They indicated that maternal or cytoplasmic effects were not found and that the alleles of the normal palmitate parent were partially dominant to those found in three of the elevated palmitate mutants.

Erickson et al. (1988) studied the inheritance of a reduced palmitate mutant, C1726, and an elevated palmitate mutant, C1727. C1726 and C1727 were developed by treating the cultivar 'Century' with EMS. C1726 has ~ 80 g kg^{-1} palmitate and C1727 has ~ 170 g kg^{-1} palmitate. They crossed each of the mutants with Century and reported that the segregation of the

F2 progeny fit the 1:2:1 model expected for two alleles at a single locus. They also crossed the two mutants and observed that the progeny segregated in a pattern consistent with recessive alleles at two independent loci. On the basis of these results, they gave the designation *fap1* to the allele which controls reduced palmitate in C1726 and *fap2* to the allele that controls elevated palmitate in C1727.

Fehr et al. (1991a) reported the inheritance of A22, which has < 70 g kg^{-1} palmitate. They crossed A22 with C1726 and obtained transgressive segregates with a lower palmitate content than either of the parents. From this study, they determined that A22 and C1726 have recessive alleles for reduced palmitate at two independent loci. A18, a line derived from the A22 X C1726 cross, had a palmitate content of < 40 g kg^{-1} and has been instrumental in the development of low saturate cultivars at Iowa State University.

In a later study, Fehr et al. (1991b) studied the genetic control of elevated palmitate in the mutants A21 and A24. A21 was developed from the treatment of 'A1937' with EMS and has ~ 200 g kg^{-1} palmitate, A24 was developed from the treatment of 'Elgin' with NMU and has a palmitate content of ~ 180 g kg^{-1}. When the two mutants were crossed, transgressive segregates were obtained in the progeny that had a palmitate content > 250 g kg^{-1}. In the genetic analysis of the two mutants, they

determined that A21 and A24 had recessive alleles for elevated palmitate at two independent loci. In the same study they reported that A21 and C1727 had been crossed to determine the relationship between the alleles for elevated palmitate of the two mutants. Their results suggested that the allele for elevated palmitate content in A21 was at the same locus or a tightly linked locus as the *fap2* allele in C1727 and designated the allele *fap2-b*. In addition, they temporally designated the allele controlling elevated palmitate in A24 as *fap?* because the relationship to the *fap1* locus in C1726 was not known.

Genetic studies of the inheritance of elevated stearate content have been reported. Graef et al. (1985) studied three elevated stearate mutants, A6, FA41545, and A81-606085, which had a range of 155 to 304 g kg^{-1} stearate. They determined that the genetic control of elevated stearate in each of the mutants was a recessive allele at a single locus. They reported that the allele fas^a in the mutant A6, and the fas^b in FA41545 were not dominant to each other, but were dominant to the *fas* allele in A81-606085.

Bubeck et al. (1989) reported the inheritance of four elevated stearate mutants and their relationship to the fas^a allele in A6. They found that three of the mutants had recessive alleles at the same locus as A6. There was one

stearate mutant that when crossed to A6 resulted in transgressive segregates with a stearate content of 336 to 365 g kg^{-1}.

Wilcox and Cavins (1985) studied the inheritance of a reduced linolenate mutant, C1640. C1640 has ~ 40 g kg^{-1} linolenate and was developed by treating Century with EMS. They crossed C1640 to Century, and the progeny had a near perfect fit to the ratio expected with two alleles at a single locus. They designated the allele controlling reduced linolenate in C1640 as *fan*.

Not all fatty ester mutants in soybeans have fit a single gene model. Graef et al. (1988) examined the genetic control of low linolenate in the mutant A5, which has 35 g kg^{-1} linolenate. They found that when A5 was crossed to high-yielding cultivars, the progeny demonstrated quantitative inheritance.

Fehr et al. (1992) found that modifying genes influence the expression of the major genes in the low linolenate mutants, A16 and A17. A16 and A17, which have 20 - 25 g kg^{-1} linolenate, were developed from the cross of A5 and an elevated palmitate mutant, A23. When A16 and A17 were crossed to Century, the F2 progeny fit a 1:14:1 ration that would indicate two major genes at independent loci. They suggested

that low linolenate in A16 and A17 is controlled by two major genes with modifying genes that have minor effects.

Fatty Ester Development in the Seed oil of Mutant Soybean Lines

There is limited information on fatty ester development in mutant lines of soybean. Fehr et al. (1971) examined the relationship of chlorophyll content and fatty ester development in two plant introductions with reduced linolenate and in two widely grown cultivars. The chlorophyll content in each of the genotypes was reduced by covering the nodes with aluminum foil before pod development. They collected covered and uncovered pods at 4-day intervals beginning at 22 DAF and analyzed the seeds for fatty ester content. They reported there were no differences in the final fatty ester content among treatments and concluded that linolenate content was not related to chlorophyll content.

Rubel et al. (1972) and Cherry et al. (1984) evaluated fatty ester development in several different cultivars. They both reported that the greatest change in fatty ester development in the seed occurred during the first half of seed formation. They also reported, that although there were some differences among cultivars, there was generally a decrease in palmitate, stearate, and linolenate accompanied by an increase in stearate and oleate.

Carver and Wilson (1984) studied triacylglycerol metabolism in a high oleate line, N78-2245, that was developed by recurrent selection. They found that oleate content in N78-2245 was 553 g kg^{-1} at 30 DAF compared with 267 g kg^{-1} for the cultivar 'Dare'. Oleate content decreased during seed development for both genotypes, however, the oleate content in N78-2245 was twofold higher than Dare at maturity.

Graef et al. (1985) studied the fatty ester development in the elevated stearate mutant, A6, and its parent, FA8077. They reported an increase in stearate content and a decrease in oleate content from 19 days after flowering (DAF) to 25 DAF in developing seeds of A6 compared with FA8077. They reported no differences between the two genotypes in the development of palmitate, linoleate, and linolenate content.

Fatty Ester Development in the Vegetative Tissues of Mutant Soybean Lines

Little is known about the relationship between the fatty ester content of the seed and other tissues of the plant for soybean genotypes selected for modified fatty ester composition. Martin and Rinne (1985) studied the relationship between the fatty ester content of roots and leaves with that of the seed oil for genotypes containing elevated stearate, elevated oleate, and reduced linolenate. They reported differences among the genotypes for fatty ester content in the

vegetative tissues, but concluded they were not consistently associated with differences in fatty ester content in the seed oil. However, they did find that the elevated stearate genotype, A6, had a significantly higher stearate content in the roots than the other genotypes in the study. In addition, they reported that the reduce linolenate genotype had the lowest linolenate content in the roots.

Wang et al. (1989) studied the relationship between the fatty ester content of roots, stems, and leaves with that of the seed oil of reduced linolenate mutants of soybean. Their results agreed with those found by Martin and Rinne in that the reduced linolenate mutants had the lowest linolenate content in the roots, and that there were no significant differences in the stems and leaves. They suggested that the mechanism for the formation of low linolenate in the roots and seed may be similar, but different from the stems and leaves.

CHAPTER 2. INHERITANCE OF REDUCED AND ELEVATED PALMITATE IN MUTANT LINES OF SOYBEAN

A paper published in the journal Crop Science

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Abstract

The saturated fatty ester content of soybean [*Glycine max* (L.) Merr.] oil may influence its use in food products. The palmitate content in the seed oil of common soybean cultivars is ≈ 110 g kg^{-1}. Soybean mutants with reduced or elevated palmitate have been developed. Previous research has shown that the alleles controlling reduced palmitate in C1726 (*fap1*) and A22 (*fapx*) were at independent loci, the alleles controlling elevated palmitate in A21 (*fap2-b*) and A24 (*fap?*) were at independent loci, and that A21 and C1727 (*fap2*) had alleles at the same locus or at tightly linked loci. The objective of this study was to determine if the *fapx* allele in A22 was at the same locus as *fap2* and if the *fap?* allele in A24 was at the same locus as *fap1*. C1726 was crossed to A21 and A24. A22 was crossed to C1727, A21, and A24. The F_2 segregation ratio and the segregation of F_3 seeds from F_2 plants indicated that A22 and A24 have alleles at different independent loci than do C1726, C1727, A21, and each other. The temporary designation of *fapx* for the allele in A22 was

replaced by the permanent designation of *fap3*, and the temporary designation of *fap?* for the allele in A24 was replaced by the permanent designation of *fap4*. The study showed that there are at least four independent loci in soybean that control palmitate content.

Introduction

The content of the saturated fatty esters in soybean oil may determine its economic value in the future. A reduction in palmitate content would improve the nutritional quality of the oil. An elevation in palmitate content could enhance its use in the production of plastic fat products, such as shortening and margarine. The palmitate content in the seed oil of commercial soybean cultivars is ≈ 110 g kg^{-1}. Soybean genotypes with < 40 g kg^{-1} and > 280 g kg^{-1} palmitate in the seed oil have been developed at Iowa State University (Fehr et al., 1991a, 1991b).

The line A18 (< 40 g kg^{-1} palmitate) was developed by crossing A22 (≈ 78 g kg^{-1} palmitate) with C1726 (≈ 85 g kg^{-1} palmitate) (Fehr et al., 1991a). C1726 has the *fap1* allele for reduced palmitate (Erickson et al., 1988). A22, previously designated A1937NMU-173, has an allele for reduced palmitate that was given the temporary designation of *fapx* because its relationship to the *fap2* locus was not known (Fehr et al.,1991a).

The line A19 (> 280 g kg^{-1} palmitate) was developed by crossing A21 (≈ 200 g kg^{-1} palmitate) with A24 (≈ 180 g kg^{-1} palmitate). A21, previously designated A1937NMU-85, has an allele (*fap2-b*) for elevated palmitate content (Fehr et al. 1991b) at the same locus or a tightly linked locus as the *fap2* allele in C1727 (Erickson et al. 1988). A24, previously designated ElginEMS-421, has an allele controlling elevated palmitate that was given the designation of *fap?* because the relationship to the *fap1* locus was not known.

The objective of this study was to determine if the *fapx* allele in A22 was at the same locus as *fap2* and if the *fap?* allele in A24 was at the same locus as *fap1*.

Materials and Methods

A21 and A22 are M4 plant selections from the treatment of 'A1937' with N-nitroso N-methyl urea (NMU), as described by Fehr et al. (1991a, 1991b). A24 is a M2 plant selection from the treatment of 'Elgin' with ethyl methanesulfonate (EMS). C1726 and C1727 were developed from the treatment of 'Century' with EMS (Erickson et al., 1988).

The following crosses and their reciprocals were made at the Agricultural Engineering and Agronomy Research Center near Ames, IA, in 1990: C1726 x A21, C1726 x A24, A22 x C1727, A22 x A21, and A22 x A24. Plants of the parents used for crossing

were identified, and selfed seeds were harvested from a node adjacent to one from which the F_1 seed was obtained. A randomized complete-block design was used for analysis of the F_1 and parent seeds. Each replicate consisted of one selfed seed of each of the parents and one seed of each of the reciprocal F_1 hybrids (Table 1). Each seed was cut into two parts with a razor blade. The part with the embryonic axis, used for planting, was approximately two-thirds of the seed. The cotyledonary part, used for fatty ester analysis, was approximately one-third of the seed. The identity of all seeds and their progeny was maintained during analysis, planting and harvest. The palmitate contents of the reciprocal F_1 and parent seeds were compared by using a standard analysis of variance for a randomized complete-block design to determine the presence of maternal effects and dominance relationships.

The part of the F_1 and parent seed containing the embryonic axis was planted at the Iowa State University breeding nursery at the Isabela Substation of the University of Puerto Rico in 1990. The seeds were planted 15 cm apart in the same order used for the analysis of the cotyledonary part of the seed. Each F_1 and parent plant was harvested individually. A random sample of 11 F_2 seeds from each of five F_1 plants for each reciprocal cross and 10 seeds of each

parent were analyzed as split seeds by the same procedure used for the F_1 seeds. The part of the seed containing the embryonic axis was planted at the Agricultural Engineering and Agronomy Research Center near Ames in 1991. The seeds were planted in rows 15 cm apart and one meter between rows. Each of the plants was harvested individually. For the genotypic evaluation of the F_2 plants, a random sample of 11 individual F_3 seeds from each of 50 random F_2 plants from each cross and four seeds from each of five individual plants of each parent were analyzed for fatty ester composition.

The palmitate contents of the parents grown in the same environments as the F_1 and F_2 plants were used to make the parental classifications. The Chi-square test with the critical value at $p = 0.05$ was used to evaluate proposed segregation ratios.

The fatty ester content of the F_1, F_2, F_3, and parent seeds was determined by gas chromatography as described by Hammond (1991). The cotyledonary part of each seed was crushed in a hydraulic press at 3.4×10^6 pascals and the oil was extracted in 1 mL of distilled hexane for 12 hours at room temperature. A 0.1-ml aliquot of the oil/hexane solution was reacted in a 1.5-ml autosampler vial with 0.5 ml of 1.0 M aqueous sodium methoxide for 30 minutes. This reaction transesterfied the oil to form fatty acid methyl esters. The

reaction was stopped by using 0.8 ml of distilled water and the methyl esters were extracted with 0.5 ml of distilled hexane. Approximately 0.2 µL of the methyl ester-hexane solution was injected into a Hewlett Packard (Avondale, PA) gas chromatograph fitted with flame detectors and 15-M Durabond-23 capillary columns (J & W Scientific, Deerfield, IL). A Hewlett Packard computer was used to control injection and convert peak areas into palmitate, stearate, oleate, linoleate, and linolenate contents.

Results and Discussion

No maternal effects for palmitate content were observed in the analysis of F_1 seeds from the reciprocal crosses (Table 1). The results indicated that the genotype of the embryo and not the genotype of the maternal plant determined its palmitate content.

No dominance effects for palmitate content were observed for any of the crosses, except for C1726 X A21. The F_1 seeds of C1726 X A21 were significantly lower in palmitate content than the midparent value, indicating partial dominance for reduced palmitate in the cross (Table 1).

The palmitate content of the parents grown in the same environment as the F_1 plants was used to evaluate the segregation among 110 F_2 seeds for each cross. The range of palmitate content of each parent was used to determine the

parental classes for each cross (Table 2). The F_2 seeds were classified as = P1, > P1 to < P2, and = P2 (Table 2). The segregation of the F_2 seeds from each cross was initially evaluated with a 1:2:1 ratio that would be expected with two alleles at one locus, and none of the crosses satisfactorily fit the ratio. When the segregation patterns were evaluated with a 1:14:1 ratio that would be expected with alleles at two independent loci, all the crosses had a satisfactory fit, except A22 X C1727 (Table 2). The lack of fit to a 1:14:1 ratio in the cross A22 X C1727 was due to the high number of F_2 seeds that had a palmitate content equal to C1727.

The palmitate content of the parents grown in the same environment as the F_2 plants was used to evaluate the segregation among F_3 seeds from each F_2 plant for each cross. The range of palmitate content for each parent was used to determine the parental classes for each cross (Tables 3 to 7). The theoretical model for the evaluation of F_3 progeny from F_2 plants was based on previous research by Fehr et al. (1991a,b). The model assumed that P1 contained an allele for reduced palmitate content, P2 contained an allele for elevated palmitate content, the alleles from the parents were at independent loci, and that the alleles at the loci had additive gene action (Tables 3 to 7). According to the model, six segregation patterns would be expected for F_3 seeds from F_2

plants. The six patterns would be: all seeds = P1; seeds = P1, > P1 and < P2; all seeds > P1 and < P2; seeds = P1, > P1 and < P2, and = P2; seeds > P1 < P2, and = P2; and all seeds = P2, with a genotypic ratio of 1:4:2:4:4:1.

The F_2 plants satisfactorily fit the expected genotypic ratio of 1:4:2:4:4:1 for all of the crosses, except A22 X A21 (Tables 3 to 7). Of the 50 random F_2 plants that were selected from the cross A22 X A21, none of them were homozygous for the A22 parental type, *Fap2-bFap2-bfap3fap3*, which could partly explain the lack of fit to the expected ratio. There were six F_2 plants in the cross, A22 X A21, with the genotype *Fap2-bFap2-bFap3Fap3* or *fap2-bfap2-bfap3fap3*, which would confirm that A22 and A21 had different alleles at two independent loci (Table 6). The results of this study indicated that A22 and A24 have alleles for palmitate content at different independent loci than C1726, C1727, A21, and each other. The temporary designation of *fapx* for the allele in A22 was replaced by the permanent designation *fap3*. The temporary designation of *fap?* for the allele in A24 was replaced by the permanent designation *fap4*.

Table 1. Mean palmitate content of F_1 seeds from reciprocal crosses and seeds of the parents.

Parent or cross	Palmitate content	Parent or cross	Palmitate content
	g kg^{-1}		g kg^{-1}
C1726	84	A22	65
C1726 X A21	131	A22 X A21	133
A21 X C1726	132	A21 X A22	136
A21	207	A21	203
LSD (0.05)†	8	LSD (0.05)	5
Midparent	146	Midparent	134
LSD (0.05)‡	8	LSD (0.05)	NS
No. of reps§	15	No. of reps	16
C1726	84	A22	67
C1726 X A24	128	A22 X A24	124
A24 X C1726	129	A24 X A22	124
A24	179	A24	182
LSD (0.05)	3	LSD (0.05)	5
Midparent	131	Midparent	124
LSD (0.05)	NS	LSD (0.05)	NS
No. of reps	14	No. of reps	14
A22	62		
A22 X C1727	129		
C1727 X A22	123		
C1727	182		
LSD (0.05)	7		
Midparent	122		
LSD (0.05)	NS		
No. of reps	14		

† Least significant difference (P = 0.05) for comparison of F_1 and parent values.

‡ Least significant difference (P = 0.05) for comparison of the midparent and F_1 values.

§ A replication consisted of one seed of each of the parents and one seed of each of the reciprocal F_1 hybrids.

Table 2. Palmitate content of parent and F_2 seeds.

Parent or cross	Palmitate content x ± SD§	range	Frequency distribution =P1	>P1 to <P2	=P2	$x^{2\dagger}$ 1:2:1	P	$x^{2\ddagger}$ 1:14:1	P
	----- g kg^{-1} -----		--------- no. --------						
C1726(P1)	85 ± 2	80-89							
A21(P2)	206 ± 4	199-213							
F_2 seeds	131 ± 28	75-205	7	102	1	80.98	<0.01	5.33	>0.05
C1726(P1)	85 ± 2	80-89							
A24(P2)	177 ± 8	167-192							
F_2 seeds	128 ± 22	85-170	4	101	3	80.38	<0.01	3.63	>0.10
A22(P1)	77 ± 4	71-87							
C1727(P2)	178 ± 5	171-190							
F_2 seeds	132 ± 31	64-203	7	88	15	40.76	<0.01	10.29	<0.01
A22(P1)	77 ± 4	71-87							
A21(P2)	206 ± 4	199-213							
F_2 seeds	141 ± 36	68-221	4	96	10	61.78	<0.01	2.62	>0.20
A22(P1)	77 ± 4	71-87							
A24(P2)	175 ± 4	167-192							
F_2 seeds	125 ± 25	71-183	8	97	5	64.29	<0.01	0.70	>0.50

†x^2 for goodness of fit to a 1:2:1 ratio for all crosses.
‡x^2 for goodness of fit to a 1:14:1 ratio for all crosses.
§ Mean plus or minus one standard deviation.

Table 3. Classification of 50 F_2 plants from the cross C1726 (*fap1fap1Fap2-bFap2-b*)X A21 (*Fap1Fap1fap2-bfap2-b*) based on the phenotypic pattern of 11 F_3 seeds from each F_2 plant. The expected F_3 phenotypic patterns are based on a model for alleles with additive gene action at two independent loci controlling palmitate content, which predicts a 1:4:2:4:4:1 F_2 genotypic ratio.

Proposed F2 genotype	Expected F_3 phenotypic pattern =P1†	>P1 to <P2	=P2	F_2 plants Expected	Observed‡
				------ no.	------
fap1fap1Fap2-bFap2-b	X			3.125	3
fap1fap1Fap2-bfap2-b or *Fap1fap1Fap2-bFap2-b*	X	X		12.500	11
fap1fap1fap2-bfap2-b or *Fap1Fap1Fap2-bFap2-b*		X		6.250	9
Fap1fap1Fap2-bfap2-b	X	X	X	12.500	7
Fap1fap1fap2-bfap2-b or *Fap1Fap1Fap2-bfap2-b*		X	X	12.500	19
Fap1Fap1fap2-bfap2-b			X	3.125	1

† P1 was C1726 (≤ 78 g kg^{-1} palmitate) and P2 was A21 (≥ 180 g kg^{-1} palmitate).

‡ Observed genotypic frequency satisfactorily fit the expected frequency based on a Chi-square test ($\chi 2 = 8.94$, $p > 0.10$).

Table 4. Classification of 50 F_2 plants from the cross C1726 (*fap1fap1Fap4Fap4*) X A24 (*Fap1Fap1fap4fap4*) based on the phenotypic pattern of 11 F_3 seeds from each F_2 plant. The expected F_3 phenotypic patterns are based on a model for alleles with additive gene action at two independent loci controlling palmitate content, which predicts a 1:4:2:4:4:1 F_2 genotypic ratio.

Proposed F2 genotype	Expected F_3 phenotypic pattern =P1[†]	>P1 to <P2	=P2	F_2 plants Expected	Observed[‡]
				------ no. ------	
fap1fap1Fap4Fap4	X			3.125	4
fap1fap1Fap4fap4 or *Fap1fap1Fap4Fap4*	X	X		12.500	16
fap1fap1fap4fap4 or *Fap1Fap1Fap4Fap4*		X		6.250	8
Fap1fap1Fap4fap4	X	X	X	12.500	3
Fap1fap1fap4fap4 or *Fap1Fap1Fap4fap4*		X	X	12.500	15
Fap1Fap1fap4fap4			X	3.125	4

[†] P1 was C1726 (≤ 80 g kg^{-1} palmitate) and P2 was A24 (≥ 154 g kg^{-1} palmitate).

[‡] Observed genotypic frequency satisfactorily fit the expected frequency based on a Chi-square test ($\chi 2 = 9.67$, $p > 0.05$).

Table 5. Classification of 50 F_2 plants from the cross A22 (*Fap2Fap2fap3fap3*) X C1727 (*fap2fap2Fap3Fap3*) based on the phenotypic pattern of 11 F_3 seeds from each F_2 plant. The expected F_3 phenotypic patterns are based on a model for alleles with additive gene action at two independent loci controlling palmitate content, which predicts a 1:4:2:4:4:1 F_2 genotypic ratio.

Proposed F2 genotype	Expected F_3 phenotypic pattern =P1[†]	>P1 to <P2	=P2	F_2 plants Expected	Observed[‡]
				------ no.	------
fap2fap2Fap3Fap3			X	3.125	6
fap2fap2Fap3fap3 or *Fap2fap2Fap3Fap3*		X	X	12.500	13
fap2fap2fap3fap3 or *Fap2Fap2Fap3Fap3*		X		6.250	8
Fap2fap2Fap3fap3	X	X	X	12.500	10
Fap3fap2fap3fap3 or *Fap2Fap2Fap3fap3*	X	X		12.500	10
Fap2Fap2fap3fap3	X			3.125	3

[†] P1 was A22 (≤ 78 g kg^{-1} palmitate) and P2 was C1727 (≥ 148 g kg^{-1} palmitate).

[‡] Observed genotypic frequency satisfactorily fit the expected frequency based on a Chi-square test ($\chi 2 = 3.90$, $p > 0.50$).

Table 6. Classification of 50 F_2 plants from the cross A22 (*Fap2-bFap2-bfap3fap3*) X A21 (*fap2-bfap2-bFap3Fap3*) based on the phenotypic pattern of 11 F_3 seeds from each F_2 plant. The expected F_3 phenotypic patterns are based on a model for alleles with additive gene action at two independent loci controlling palmitate content, which predicts a 1:4:2:4:4:1 F_2 genotypic ratio.

Proposed F2 genotype	Expected F_3 phenotypic pattern =P1[†]	>P1 to <P2	=P2	F_2 plants Expected	Observed[‡]
				------ no. ------	
fap2-bfap2-bFap3Fap3			X	3.125	6
fap2-bfap2-bFap3fap3 or *Fap2-bfap2-bFap3Fap3*		X	X	12.500	16
fap2-bfap2-bfap3fap3 or *Fap2-bFap2-bFap3Fap3*		X		6.250	6
Fap2-bfap2-bFap3fap3	X	X	X	12.500	6
Fap2-bfap2-bfap3fap3 or *Fap2-bFap2-bFap3fap3*	X	X		12.500	16
Fap2-bFap2-bfap3fap3	X			3.125	0

[†] P1 was A22 (≤ 82 g kg^{-1} palmitate) and P2 was A21 (≥ 180 g kg^{-1} palmitate).

[‡] Observed genotypic frequency did not satisfactorily fit the expected frequency based on a Chi-square test ($\chi 2 = 11.75$, $p > 0.01$).

Table 7. Classification of 50 F_2 plants from the cross A22 (*fap3fap3Fap4Fap4*)X A24 (*Fap3Fap3fap4fap4*) based on the phenotypic pattern of 11 F_3 seeds from each F_2 plant. The expected F_3 phenotypic patterns are based on a model for alleles with additive gene action at two independent loci controlling palmitate content, which predicts a 1:4:2:4:4:1 F_2 genotypic ratio.

Proposed F2 genotype	Expected F_3 phenotypic pattern =P1[†]	>P1 to <P2	=P2	F_2 plants Expected	Observed[‡]
				------ no. ------	
fap3fap3Fap4Fap4	X			3.125	3
fap3fap3Fap4fap4 or *Fap3fap3Fap4Fap4*	X	X		12.500	9
fap3fap3fap4fap4 or *Fap3Fap3Fap4Fap4*		X		6.250	6
Fap3fap3Fap4fap4	X	X	X	12.500	16
Fap3fap3fap4fap4 or *Fap3Fap3Fap4fap4*		X	X	12.500	12
Fap3Fap3fap4fap4			X	3.125	4

[†] P1 was A22 (≤ 85 g kg^{-1} palmitate) and P2 was A24 (≥ 150 g kg^{-1} palmitate).

[‡] Observed genotypic frequency satisfactorily fit the expected frequency based on a Chi-square test ($\chi 2 = 2.24$, $p > 0.50$).

CHAPTER 3. FATTY ESTER DEVELOPMENT IN REDUCED AND ELEVATED PALMITATE LINES OF SOYBEAN

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Abstract

Genetic modifications of soybean [*Glycine max* (L.) Merr.] have been made to reduce and elevate palmitate content in the seed oil. One objective of this study was to evaluate the changes in palmitate content that occur during seed development of reduced and elevated palmitate soybean genotypes. A second objective was to determine the relationship between the palmitate content of the leaves, stems, roots, and seeds of the soybean genotypes. The genotypes evaluated were `Elgin 87,' with a normal palmitate content of 110 g kg^{-1}; A18, a reduced palmitate line with < 45 g kg^{-1}; and A19, an elevated palmitate line with > 250 g kg^{-1}. Seeds of each of the lines were harvested at 2-d intervals from 15 to 39 d after flowering (DAF) and 4-d intervals from 39 DAF until maturity during 1991 and 1992. Significant differences among the lines for palmitate content were observed as early as 19 DAF. The increase in palmitate in A19 was accompanied by a decrease in oleate and linoleate content. The decrease in palmitate in A18 was accompanied by an

increase in oleate content. There was a relationship between the palmitate content in the seed oil and that of the roots, leaves, and stems for each of the lines. A18, which had the lowest palmitate content in the seed oil, had the lowest palmitate content in roots, leaves, and stems. A19, which had the highest palmitate content in the seed oil, had the highest palmitate content in the vegetative tissues studied. The results indicated that the mutant alleles in A18 and A19 are constitutively expressed.

Introduction

Soybean [*Glycine max* (L.) Merr.] genotypes with reduced and elevated palmitate content have been developed to improve the seed oil for food and industrial applications (Schnebly et al., 1994). The palmitate content of current soybean cultivars is 110 g kg^{-1}. A genotype with < 45 g kg^{-1} palmitate was developed by combining the *fap1* and *fap3* alleles for reduced palmitate, and a genotype with > 250 g kg^{-1} was developed by combining the *fap2-b* and *fap4* alleles for elevated palmitate (Fehr et al., 1991a,b; Schnebly et al., 1994).

It would be useful in a breeding program to be able to distinguish between normal, reduced, and elevated palmitate genotypes before the flowering of a plant is complete or before seed is mature. Selection before the termination of

flowering would make it possible to select and cross individuals during the same season. Selection before maturity could reduce the number of plants harvested and threshed and could shorten the time between harvest, selection, and replanting of individuals. The objectives of our study were to determine the stage of seed development at which genotypes with different palmitate contents can be distinguished and to compare the palmitate content of roots, stems, leaves, and mature seeds.

The fatty ester content of elevated and normal stearate genotypes of soybean during seed development was evaluated by Graef et al. (1985). They were able to differentiate the elevated and normal stearate genotypes at 19 to 25 d after flowering based on their stearate and oleate content. They did not observe significant differences between the two genotypes in the development of palmitate, linoleate, and linolenate content.

The fatty ester content of plant tissue and seed oil has been evaluated for soybean genotypes with altered stearate, oleate, and linolenate. Martin and Rinne (1985) studied genotypes with elevated stearate, elevated oleate, and reduced linolenate. They reported differences among the genotypes for fatty ester content in the vegetative tissues, but those differences were not consistently associated with composition

of the seed oil. They reported, however, that the genotype with elevated stearate in the seed had the highest stearate content in the roots and the genotype with reduced linolenate in the seed had the least linolenate in the roots. Wang et al. (1989) found that mutants with reduced linolenate had less linolenate in the roots than did normal genotypes, but linolenate content of stems and leaves did not significantly differ among genotypes.

Materials and Methods

The genotypes used for the study were A18 (< 45 g kg^{-1} palmitate) (Fehr et al., 1991a), A19 (> 250 g kg^{-1} palmitate) (Fehr et al., 1991b), and `Elgin 87' (110 g kg^{-1} palmitate). Each of the genotypes was grown in 10 rows, 3.4 m long and 1 m apart, at the Agricultural Engineering and Agronomy Research Center near Ames, IA during 1991 and 1992. The lines were planted on 11 May 1991 and 12 May 1992. The seeding rate was 26 seeds m^{-1}. When the plants of each genotype reached stage R2, 500 flowers were tagged at the third, fourth, or fifth node from the top of the mainstem of the plants. Plants at R2 have an open flower at one of the two uppermost nodes on the mainstem with a fully developed leaf (Fehr and Caviness, 1977). Any buds or old flowers were removed to obtain only one tagged flower at each node. The age of the seeds in each

pod was expressed as the number of days between opening of the flower and harvest of the pod (DAF).

Seeds were harvested at 2-d intervals from 15 to 39 DAF and at 4-d intervals from 39 DAF to maturity (71 DAF). On each of the 21 sampling-dates, 15 pods of each genotype were removed from the plants and taken to the laboratory. The seeds were removed from the pods and the weights of three 10-seed samples of each genotype were determined. Each 10-seed sample was placed in a separate test tube and frozen at -80°C until all samples from the 21 dates had been collected.

The leaf, stem, and root tissues were collected at stages V2 and R6. Plants at V2 have a fully developed leaf at a node above the unifoliolate nodes, and at R6 they have a pod that contains a green seed that fills the pod cavity at one of the four uppermost nodes on the mainstem with a fully developed leaf. At each stage, 15 random plants from each genotype were harvested. For each plant at V2, the trifoliolates were cut into 1-cm^2 pieces and the roots and stems were cut into 1-cm lengths and stored at -80°C. For each plant at R6, five random trifoliolates were obtained and cut into 1-cm^2 pieces, the roots and stems were cut into 1-cm lengths, and a random 5-g sample of each of the tissues from each plant was stored at -80°C.

To initiate fatty-ester analysis, each 10-seed sample was freeze-dried for 24 hr and the dry weight was recorded. The samples were ground to a fine powder with a Mitey Mill (Sturdee Health Products, Island Park, NY). A chloroform-methanol solution (2:1, v/v) was added to each sample and the chloroform-methanol solution was removed after 12 hr. A volume of water equal to 1/5 the volume of the chloroform-methanol solution was added and mixed with a Genie 2 Vortex (Fisher Scientific, Bohemia, NY). This caused the solvent to separate into two layers. The lower chloroform layer was collected, and the chloroform was evaporated. The residual lipids were dissolved in 2 µL of hexane; 1 mL of the hexane solution was transferred into a 1.5 mL autosampler vial and evaporated to about 0.2 mL, and the oil/hexane solution was reacted with 0.5 mL of 1.0 M aqueous sodium methoxide for 30 min. The reaction was stopped by adding 0.8 mL of distilled water. The methyl esters were extracted with 0.5 mL of distilled hexane. Approximately 0.2 mL of the methyl ester-hexane solution was injected into a Hewlett Packard (Avondale, PA) gas chromatograph fitted with flame detectors and 15-M Durabond-23 capillary columns (J & W Scientific, Deerfield, IL). A Hewlett Packard computer was used to control injection and convert peak areas into palmitate, stearate, oleate, linoleate, and linolenate contents.

The data from the seed samples were analyzed in three replications of a split-plot design. Soybean genotypes were whole plots and sampling dates were subplots. For the analysis of variance, genotypes and sampling dates were considered to be fixed effects and years were considered random effects. The leaf, stem, and root tissue were analyzed separately in three replications of a randomized complete-block design. A replication consisted of a sample of tissue from each stage for the three genotypes. For the analysis of variance, genotypes and stages were considered to be fixed effects and years was considered a random effect.

Results

Seeds

The dry weight of the seeds, when sampling began at 15 DAF, was only about 1% of the weight of mature seeds at 71 DAF for the three genotypes (Table 1). The maximum fresh weight occurred at 43 DAF for A18 and at 51 DAF for Elgin 87 and A19, after which the seeds began to lose moisture as they began to mature. The dry weights of the three genotypes at 55 DAF were not significantly different from the weights at 71 DAF.

The differences among genotypes for palmitate content were significant ($P<0.05$) at 15 DAF in 1991, 17 DAF in 1992, and 19 DAF for the 2-yr mean (Table 2). From 17 DAF until maturity, A19 had the highest, A18 had the lowest, and Elgin

87 had an intermediate palmitate content at each sampling date in both years. The palmitate content of A18 and Elgin 87 was not as high at any sampling date as that of A19 in both years. Beginning at 15 DAF in 1991 and 17 DAF in 1992, the palmitate content of A18 was less than that of Elgin 87 at every sampling date.

The pattern of palmitate synthesis during seed development differed among the three genotypes. For A18, the mean palmitate content across years was highest at 15 DAF and decreased steadily until 39 DAF when it reached the content of mature seed. Across years, the mean palmitate content of Elgin 87 did not consistently decrease until 27 DAF. At 37 DAF, the palmitate content of seeds of Elgin 87 was the same as in the mature seeds. Averaged across years, there was no significant difference among sampling dates for the palmitate content of A19.

There was no significant difference among the three genotypes for the stearate content of mature seed, and the pattern of stearate synthesis was similar among the genotypes (Table 3). Stearate content began to decrease at about 21 DAF and was similar to that of mature seed at about 31 DAF.

Oleate content was significantly different among genotypes (Table 3). At all of the sampling dates, A19 had the least, Elgin 87 had intermediate, and A18 had the least

oleate content. There was a lower oleate content in seeds at 15 DAF than at maturity for A18 and Elgin 87. For A19, the oleate content was similar across all sampling dates.

The linoleate content of seeds at 15 DAF was less than in mature seeds for the three genotypes. Across sampling dates, the linoleate contents of A18 and Elgin 87 were similar and higher than that of A19. Linolenate content was higher at 15 DAF than in mature seeds for all the genotypes, and there were no significant differences among the genotypes across the sampling dates.

Vegetative tissue

The palmitate contents of vegetative tissues for the three genotypes had the same relative ranking as was observed in their mature seeds (Tables 2 and 4). A18 had the lowest, Elgin 87 had intermediate, and A19 had the highest palmitate contents, except for leaves at R6 in 1992. At least two of the three genotypes were significantly different from each other in each of the tissues at both stages for the individual years. The differences among genotypes for palmitate content were more pronounced at V2 than at R6 (Table 4). For example, there was a greater difference between A18 and A19 in each of the vegetative tissues at V2 than at R6. There were significant differences among the three tissues for mean palmitate content. The roots and stems had a greater

palmitate content than that observed in the leaves. The range among genotypes was greatest in the stems, intermediate in the roots, and least in the leaves for both stages and years.

Mean stearate content differed significantly among genotypes in the leaf tissue at both stages and in the stems at V2, but the rankings of the genotypes were not the same as in the mature seeds (Tables 3 and 5). A18 had the lowest stearate in the seeds and the highest stearate in the leaf and stem tissues compared with the other two genotypes. Differences among genotypes in the roots were not significant at either developmental stage.

There were no significant differences in oleate content in any of the vegetative tissues at either stage, except for the leaf tissue at V2 (Table 4). The ranking of the oleate content of the genotypes in the leaf tissue at V2 was not the same as in the mature seeds (Table 3).

The relative ranking of the three genotypes for linoleate content was consistent among the tissues for both stages of development (Table 5). A18 had the highest linoleate content in the vegetative tissues, whereas A18 and Elgin 87 did not differ in the content of the seeds. A19 had the lowest linoleate content in the vegetative tissues and the seeds compared with the other two genotypes.

The genotypes could not be consistently differentiated for linolenate content in the three tissues (Table 5). The lack of significant differences among genotypes in the vegetative tissues was similar to that observed in the seeds (Table 3).

Discussion

One objective of our study was to determine the earliest stage of seed development at which genotypes with normal, reduced, or elevated palmitate could be differentiated. In both years of the study, differences were apparent at 17 DAF and remained throughout seed development. If genotypes are to be compared during seed development, it would be best to tag flowers as was done in our study and harvest seeds at the same DAF for analysis. When the differences in palmitate content among genotypes are as large as in this study, however, the age of the seed is not critical from about 23 DAF until maturity. Selection of seeds of similar size from all genotypes to be compared should be sufficient for fatty-ester analysis. A second objective was to determine the feasibility of comparing genotypes for palmitate content by the analysis of vegetative tissue. Our results indicated that it would be possible to select leaf tissue at V2 and analyze it before flowering to select genotypes for crossing or other purposes.

Roots or stems could also be used for analysis, but their removal would cause more damage to the plant.

The effectiveness of selection of individuals based on the analysis of immature seeds or vegetative tissue would depend on the magnitude of the differences in palmitate content among them. Our results indicate that it would be possible to select the extremes in a population. Additional research will be necessary to determine how accurately genotypes can be differentiated that are more similar in palmitate content, particularly when leaf tissue is sampled at an early vegetative stage.

The consistent ranking of the three genotypes for palmitate content in the three vegetative tissues and in the seed indicated that the mutant alleles in A18 and A19 are constitutively expressed. The influence of their expression on other characteristics of the plant should be monitored to identify effects that could be detrimental if cultivars are developed and produced commercially. The challenge will be to differentiate between pleiotropic effects of the mutant alleles and effects on performance caused by genes closely linked to the mutant alleles (Ndzana et al., 1994; Horejsi et al., 1994; Hartmann et al., 1995a; Hartmann et al., 1995b).

Table 1. Mean fresh and dry weight of seeds of A18, Elgin 87, and A19 averaged over two years for different numbers of days after flowering.

Days after flowering	A18		Elgin 87		A19	
	Fresh	Dry	Fresh	Dry	Fresh	Dry
	mg seed^{-1}					
15	5 ± 1[1]	2 ± 1	3 ± 1	1 ± 1	3 ± 1	1 ± 1
17	13 ± 4	3 ± 1	5 ± 1	2 ± 1	7 ± 1	3 ± 1
19	24 ± 6	5 ± 2	8 ± 2	3 ± 1	17 ± 3	4 ± 1
21	34 ± 4	7 ± 2	22 ± 4	5 ± 2	28 ± 2	6 ± 1
23	74 ± 22	17 ± 6	38 ± 4	7 ± 2	52 ± 8	9 ± 1
25	111 ± 21	22 ± 4	70 ± 6	13 ± 1	76 ± 5	15 ± 2
27	152 ± 28	35 ± 8	108 ± 11	21 ± 2	112 ± 14	25 ± 4
29	184 ± 37	46 ± 12	131 ± 17	28 ± 5	134 ± 19	33 ± 5
31	227 ± 35	61 ± 14	169 ± 13	41 ± 4	192 ± 10	52 ± 4
33	268 ± 25	81 ± 10	221 ± 9	60 ± 3	222 ± 9	63 ± 2
35	294 ± 23	88 ± 10	241 ± 8	73 ± 4	251 ± 13	82 ± 7
37	336 ± 20	114 ± 10	284 ± 8	87 ± 5	279 ± 5	87 ± 3
39	414 ± 46	130 ± 15	315 ± 7	102 ± 5	284 ± 6	93 ± 5
43	447 ± 26	154 ± 14	367 ± 8	126 ± 7	339 ± 13	111 ± 9
47	469 ± 9	177 ± 12	413 ± 7	154 ± 4	377 ± 11	138 ± 5
51	411 ± 32	172 ± 20	448 ± 16	173 ± 10	418 ± 10	162 ± 7
55	374 ± 43	209 ± 3	445 ± 12	187 ± 9	398 ± 19	174 ± 6

Table 1. Continued.

Days after flowering	A18		Elgin 87		A19	
	Fresh	Dry	Fresh	Dry	Fresh	Dry
	mg seed^{-1}					
59	337 ± 52	186 ± 8	428 ± 12	202 ± 6	377 ± 15	182 ± 3
63	289 ± 11	185 ± 5	326 ± 17	185 ± 3	296 ± 16	179 ± 3
67	212 ± 7	186 ± 4	269 ± 5	197 ± 3	244 ± 6	183 ± 3
71	207 ± 3	185 ± 3	220 ± 4	190 ± 6	202 ± 2	171 ± 2

[1]Standard error of the mean.

Table 2. Mean palmitate-content of seeds of A18, Elgin 87, and A19 at different numbers of days after flowering during two years at Ames, IA.

Days after flowering	1991			1992			1991-92		
	A18	Elgin 87	A19	A18	Elgin 87	A19	A18	Elgin 87	A19
	g kg^{-1}								
15	125	215	284	127	103	246	126	157	265
17	144	223	275	82	122	232	113	173	254
19	119	203	286	83	128	217	101	166	251
21	100	207	308	88	128	268	94	168	288
23	66	191	281	83	125	275	75	158	278
25	62	169	288	79	186	266	70	178	277
27	53	141	280	71	153	280	62	147	280
29	50	136	283	62	139	286	56	137	284
31	49	132	286	61	130	288	55	131	287
33	45	126	286	54	124	287	49	124	286
35	43	126	279	49	119	278	46	122	278
37	48	118	282	47	114	277	48	116	280
39	41	116	273	45	110	283	43	113	278
43	40	109	266	44	109	284	42	109	275
47	41	113	271	42	107	274	42	110	272
51	44	114	265	40	104	268	42	110	267
55	42	114	262	44	108	266	43	111	264
59	42	116	263	44	106	261	43	111	262

Table 2. Continued.

Days after flowering	1991			1992			1991-92		
	A18	Elgin 87	A19	A18	Elgin 87	A19	A18	Elgin 87	A19
	----------				g kg^{-1}		----------		
63	42	114	261	45	108	253	43	111	257
67	42	118	262	45	114	251	43	116	256
71	41	115	264	48	117	247	45	116	255
$LSD_{0.05}$ within lines	19	21	19	12	22	16	27	57	31
$LSD_{0.05}$ among lines			19			15			65

Table 3. Mean fatty-ester content of seeds of three genotypes averaged over two years for different numbers of days after flowering.

Days after flowering	Stearate			Oleate			Linoleate			Linolenate		
	A18	Elgin 87	A19	A18	Elgin 87	A19	A18	Elgin 87	A19	A18	Elgin 87	A19
	g kg^{-1}											
15	70	88	71	241	184	144	414	440	376	148	128	143
17	79	83	83	276	177	145	401	397	343	130	170	175
19	53	86	61	254	158	145	461	392	315	157	198	228
21	54	69	53	218	153	118	441	400	342	192	210	198
23	46	59	44	273	169	138	440	400	334	166	213	206
25	44	48	42	256	175	144	477	420	383	153	179	153
27	41	44	37	258	177	134	495	451	395	145	181	153
29	42	43	36	272	199	141	488	443	384	142	177	154
31	41	40	34	292	209	140	472	466	395	141	154	143
33	34	39	34	282	215	140	513	478	399	121	142	140
35	35	39	37	284	209	145	518	493	411	116	136	129
37	36	46	42	299	236	156	504	480	400	113	122	123
39	37	46	39	343	237	159	474	491	402	102	113	122
43	34	44	38	333	228	149	492	511	417	98	107	120
47	35	44	41	333	220	138	502	523	437	88	102	111
51	34	43	42	317	220	151	516	523	437	90	103	103
55	34	42	41	321	212	145	518	537	447	84	97	103

Table 3. Continued

Days after flowering	Stearate			Oleate			Linoleate			Linolenate		
	A18	Elgin 87	A19	A18	Elgin 87	A19	A18	Elgin 87	A19	A18	Elgin 87	A19
	g kg^{-1}											
59	32	41	40	317	208	145	526	545	453	81	95	99
63	31	41	38	321	206	139	520	544	460	84	97	106
67	32	42	39	307	202	145	533	544	456	84	95	104
71	32	41	39	300	213	146	538	538	459	86	92	100
$LSD_{0.05}$ within genotypes	23	38	33	65	62	27	67	98	75	35	68	54
$LSD_{0.05}$ among genotypes			34			137			117			82

Table 4. Palmitate content of vegetative tissues of three genotypes during two years.

Tissue / Genotype	1991		1992		1991-92	
	V2	R6	V2	R6	V2	R6
	g kg^{-1}					
Root						
A18	210	202^{NS}	202	186^{NS}	207	194^{NS}
Elgin 87	247	217**	229	218^{NS}	237	217**
A19	286	242**	284	228**	285	234**
$LSD_{0.05}$	10	10	31	31	14	14
Stem						
A18	181	149**	170	146^{NS}	176	148**
Elgin 87	225	184**	230	182**	228	183**
A19	273	215**	263	194**	268	204**
$LSD_{0.05}$	8	8	32	32	15	15
Leaf						
A18	118	115^{NS}	107	123^{NS}	112	119^{NS}
Elgin 87	137	117**	132	144^{NS}	132	131^{NS}
A19	182	142**	174	141*	178	142*
$LSD_{0.05}$	8	8	16	16	24	24^{NS}
$LSD_{0.05}$ among tissues	8	8	24	24	17	17

*, ** Differences between stages significant at the 5% and 1% probability levels, respectively.

NS Differences between means not significant at the 5% probability level.

Table 5. Mean fatty ester content of vegetative tissues of three genotypes at two developmental stages averaged over two years.

Tissue / Genotype	Stearate		Oleate		Linoleate		Linolenate	
	V2	R6	V2	R6	V2	R6	V2	R6
	g kg^{-1}							
Root								
A18	73	75^{NS}	208	136^{NS}	374	431^{NS}	140	165^{NS}
Elgin 87	64	72^{NS}	163	207^{NS}	347	340^{NS}	187	164^{NS}
A19	73	68^{NS}	165	157^{NS}	300	327^{NS}	176	213^{NS}
$LSD_{0.05}$	20^{NS}	20^{NS}	114^{NS}	114^{NS}	74^{NS}	74	54^{NS}	54
Stem								
A18	99	68**	101	57^{NS}	313	379**	310	349^{NS}
Elgin 87	96	65**	95	51^{NS}	290	353**	292	348^{NS}
A19	87	60**	91	41^{NS}	266	330**	288	363^{NS}
$LSD_{0.05}$	8	8^{NS}	71^{NS}	71^{NS}	34	34	81^{NS}	81^{NS}

Table 5. Continued.

Tissue / Genotype	Stearate		Oleate		Linoleate		Linolenate	
	V2	R6	V2	R6	V2	R6	V2	R6
	g kg^{-1}							
Leaf								
A18	55	50^{NS}	34	35^{NS}	111	92^{NS}	688	703^{NS}
Elgin 87	46	42^{NS}	22	25^{NS}	93	70*	707	731^{NS}
A19	45	42^{NS}	21	24^{NS}	80	67^{NS}	676	726*
$LSD_{0.05}$	6	6	11	11	21	21	34^{NS}	34^{NS}
$LSD_{0.05}$ among tissues	10	10	63	63	39	39	47	47

*, ** Differences between stages significant at the 5% and 1% probability levels, respectively.

NS Differences among means not significant at the 5% probability level.

GENERAL CONCLUSIONS

The objective of the inheritance study presented in Chapter 2 was to determine if the *fapx* allele for reduced palmitate in A22 was at the same locus as *fap2* in C1727 and if the *fap?* allele in A24 for elevated palmitate was at the same locus as *fap1*. The F_2 segregation ratios and the segregation of F_3 seeds from the F_2 plants indicated that A22 and A24 have alleles at different independent loci than those of C1726, C1727, and A21. The temporary designation of *fapx* for the allele in A22 was replaced by the permanent designation of *fap3*, and the temporary designation of *fap?* for the allele in A24 was replaced by the permanent designation of *fap4*. The study showed that there are at least four independent loci in soybean that control palmitate content.

The objectives of the development study presented in Chapter 3 were to (a) determine the earliest stage of seed development at which genotypes with normal, reduced, and elevated palmitate could be differentiated and (b) determine the relationship between the fatty ester content of the seed and that of the vegetative tissues of the three palmitate genotypes. It was determined that differences among reduced, elevated, and normal palmitate lines can be detected as early as 17 DAF and remain throughout seed development. It would be

useful to determine the range of palmitate content that could be detected in seeds in segregating populations between either reduced or elevated palmitate lines and normal soybean cultivars. This would enable a breeder to screen the immature F2 seeds from F1 plants and discard plants with undesirable phenotypes earlier in the growing season.

Significant differences for palmitate content of roots, stems, and leaves were observed among the reduced, elevated and normal palmitate genotypes as early as V2, the first-trifoliate leaf stage. This would enable the breeder to sample leaf tissue before flowering to determine which plants could be used for crossing. It also indicated that the genes for reduced, elevated, and normal palmitate are constitutively expressed.

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